

# An Innovative Analysis Method for Fragrance of Sake Using an Introducing Device for Volatile Compounds Combined with DART-MS

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## Introduction

Sake is a Japanese national liquor, and many kinds are brewed for different flavor notes, such as fruits, flowers, herbs, and spices.

Additionally, Sake can be served in a variety of vessels (i.e. cups): the Ochoko, the Masu, the Sakazuki, or a wineglass, allowing one to enjoy different varieties of scents, depending on the cup.



**The purpose of this work :**  
to empirically visualize the differences of Sake fragrance, per cup-type.

## Materials and Methods

### Materials :

Hakutsuru Daiginjo (Sake)  
Four cup-types (i.e., Choko, Daichoko, Champagne glass, wineglass)



Fig. 1 Hakutsuru Daiginjo (Sake)

Fig. 2 Cups

### Analytical methods :

**HS-GC/MS(EI)**  
Head space: 60 °C, 30 min  
Column: Agilent DB-WAX (30 m x 0.25 mm id, 0.25 μm)  
Oven: 40 °C, 2 min – (5 °C/min) – 220 °C – (30 °C/min) – 250 °C, 5 min  
Total 44 min  
Sample: 5 mL (20mL vial)

**SPME-GC/MS(EI)**  
SPME: Acrylate SPME arrow (CTC Analytics), 60 °C, 30 min  
Column: Agilent HP-5ms (30 m x 0.25 mm id, 0.25 μm)  
Oven: 40 °C, 1 min – (10 °C/min) – 250 °C, 1.5 min  
Total 23.5 min  
Sample: 5 mL (20mL vial)

**GC/MS**  
Autosampler: PAL RTC (CTC Analytics)  
GC/MS: 7890GC/5977BMSD (Agilent)

Volatimeship: Open-type, introducing tube 150 °C  
Detector: DART Ionization (ionization gas: He, 400 °C)  
Q-TOF, Mass range  $m/z$  50 – 2000  
Sample: Sake (30 mL) was added into a cup.

Procedure:  
Data acquisition via the mass spectrometer were began and the first 30 sec measured background.  
Next, the cup was placed at the introducing tube of the Volatimeship, where the position of the introducing tube approximates the position of human nose where the human are drinking.

**Volatimeship DART-MS**  
Closed-chamber interface device : Volatimeship (BioChromato)  
Ion source : DART™-SVP (IonSense)  
MS Spectrometer : compact (Bruker)

## Results and discussions

### Qualitative analysis of Sake Fragrance

- Ethyl caproate, one of the characteristic fragrances of sake was detected by all analytical methods.
- For GC/MS, unknown peaks not consistent with spectral library were extremely difficult to analyze. On the other hands, Volatimeship DART-MS enabled analysis elemental composition by using accurate mass spectra.

Table. Detected compounds from Sake Fragrance

	Compounds	Mw	HS-GC/MS	SPME-GC/MS	Volatimeship DART-MS
1	Ethyl butyrate	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	116.1	Detected	
2	2-Methyl-1-propanol	C <sub>4</sub> H <sub>10</sub> O	74.1	Detected	
3	Isoamyl acetate	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub>	130.2	Detected	Detected
4	Isoamyl alcohol	C <sub>5</sub> H <sub>12</sub> O	88.2	Detected	Detected
5	Ethyl caproate	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	144.2	Detected	Detected
6	Ethyl octanoate	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	172.3	Detected	Detected
7	Ethyl caprate	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200.3	Detected	Detected
8	Phenethyl alcohol	C <sub>8</sub> H <sub>10</sub> O	122.16		Detected

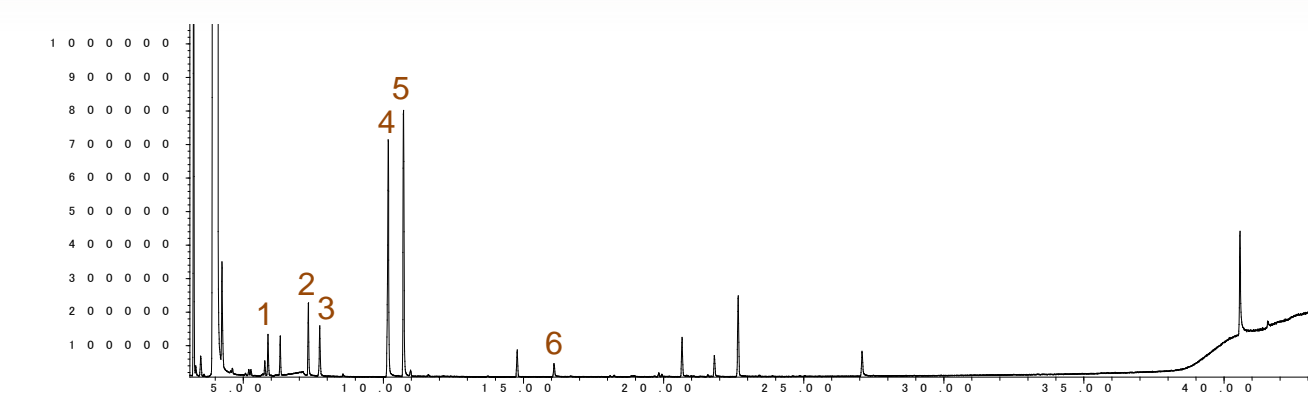
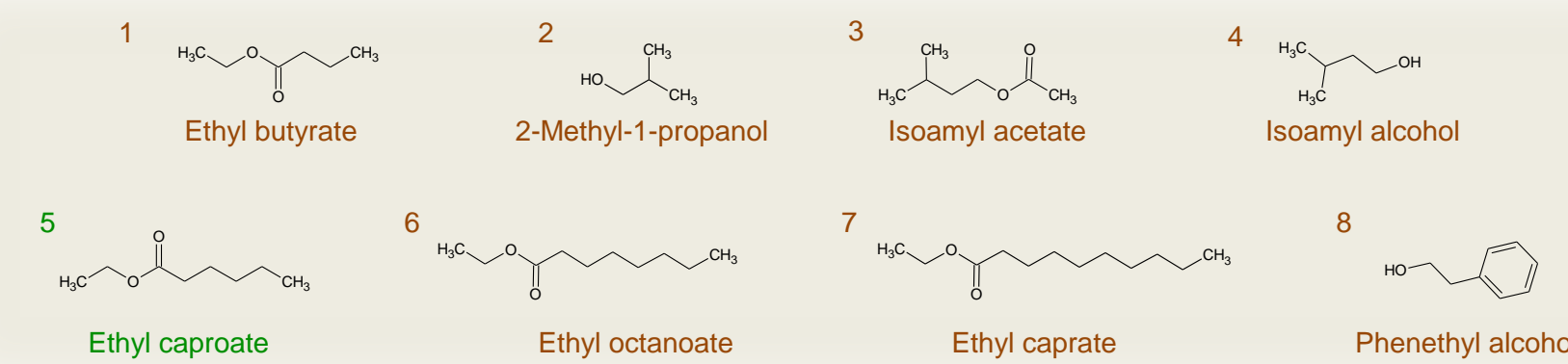


Fig. 3 TIC of Sake using HS-GC/MS

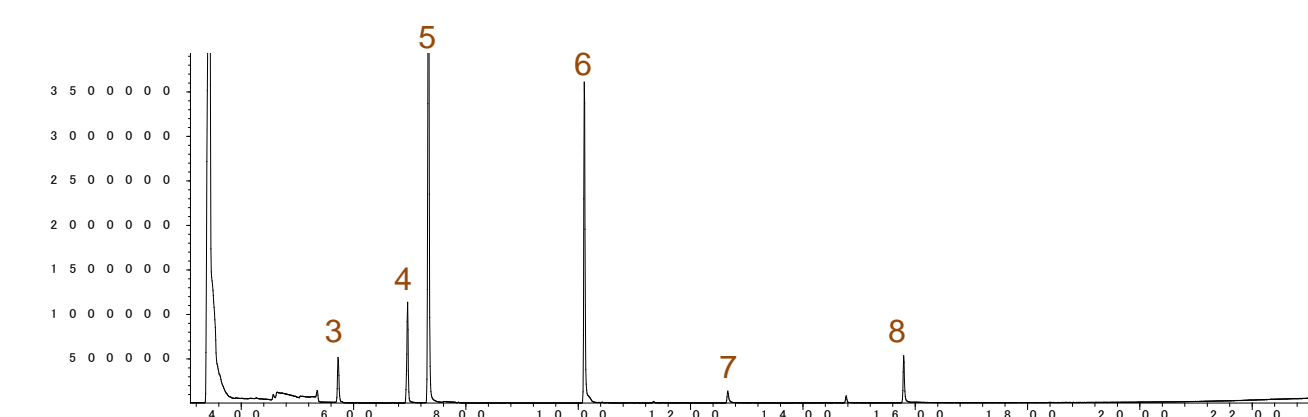


Fig. 4 TIC of Sake using SPME-GC/MS

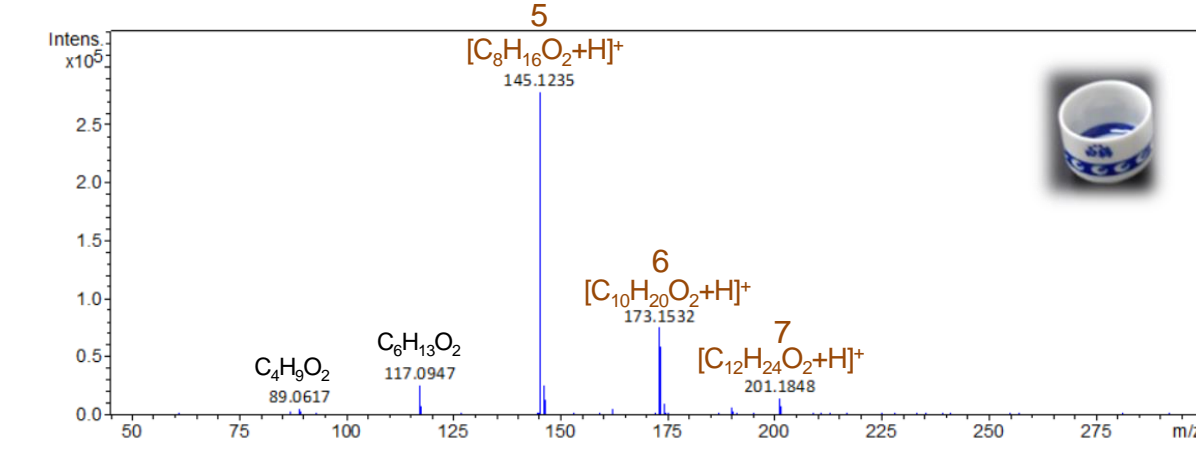


Fig. 5 Mass spectrum of Sake in the Ochoko using Volatimeship DART-MS

### Realtime Monitoring of Sake Fragrance

- Ethyl caproate was mainly detected as a protonated ion using DART ionization. An ammonium adducted ion was also detected (Fig. 6).
- EIC shows that the volatile compounds of Sake were immediately detected by mass spectrometer, as the Sake in the cups was placed at the introducing tube of the Volatimeship, and also shows the volatilization behavior of ethyl caproate (Fig. 7).
- It recognizes that the volatilization behavior was different depending on the cups. Especially, the wineglass not only shows the sharpest increase but it also shows best sustainability (Fig. 7). Moreover, these analysis results supported the results of sensory evaluation.

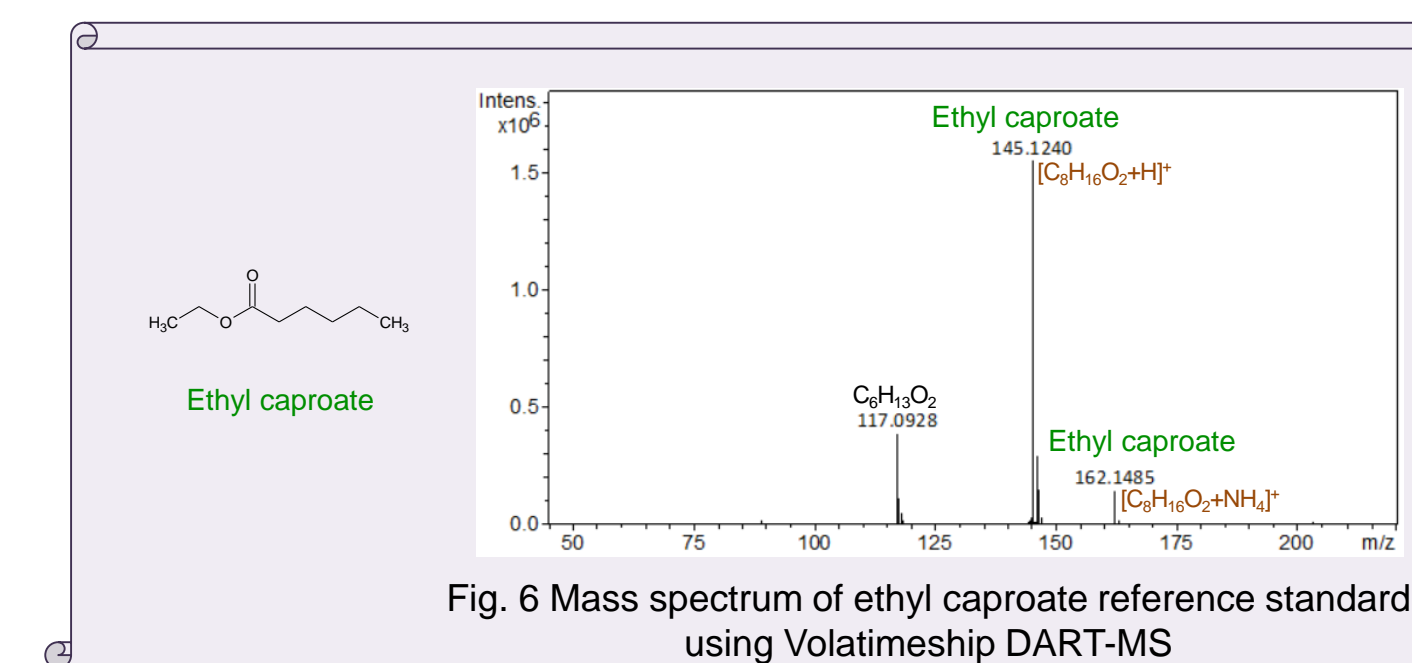


Fig. 6 Mass spectrum of ethyl caproate reference standard using Volatimeship DART-MS

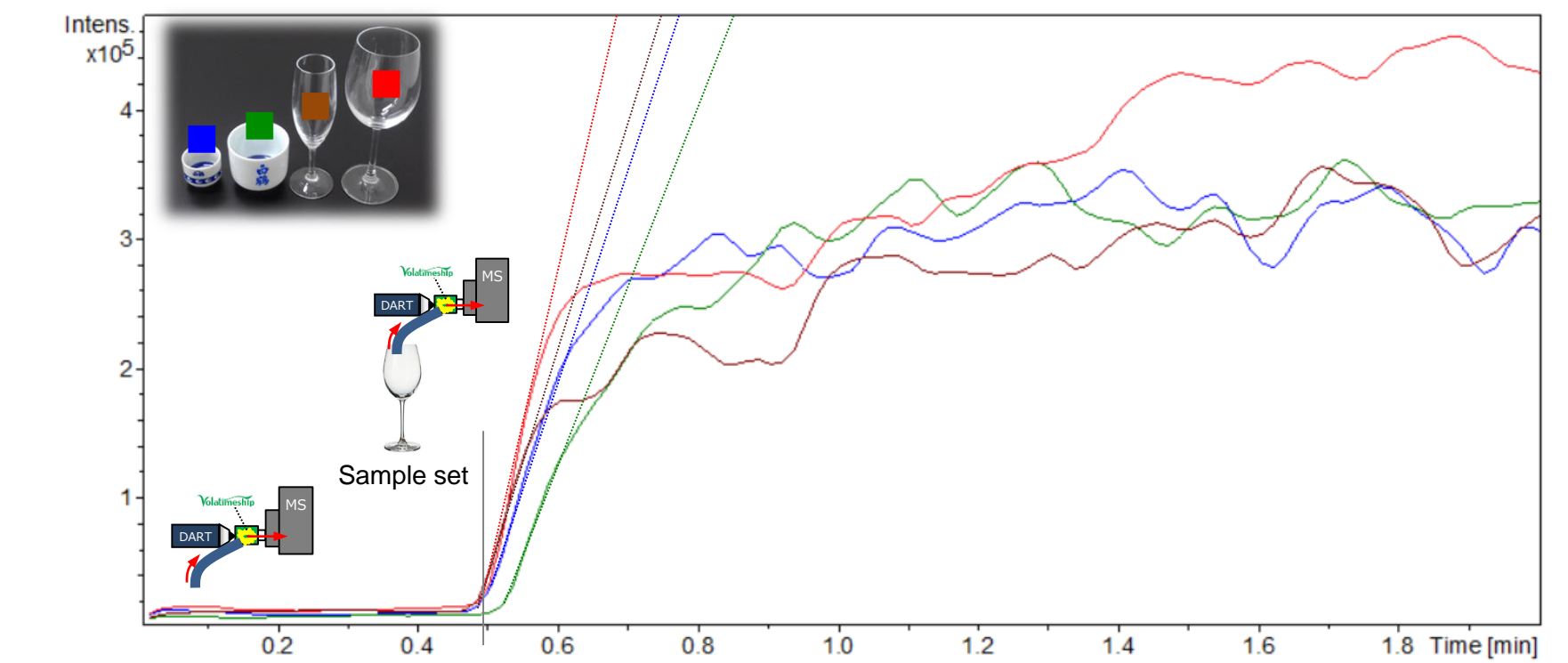


Fig. 7 EIC ( $m/z$  145.12±0.01) of Sake in the four type of cups using Volatimeship DART-MS

- The detected compounds from Sake in cups were different depending on the cups, especially for wine glass and champagne glass, there were some compound (ex. C<sub>12</sub>H<sub>26</sub>O<sub>3</sub>) that were not detected from others (Fig. 8).
- Additionally, its volatilization behavior was different depending the cups (Fig. 9), and it may support the results of sensory evaluation.

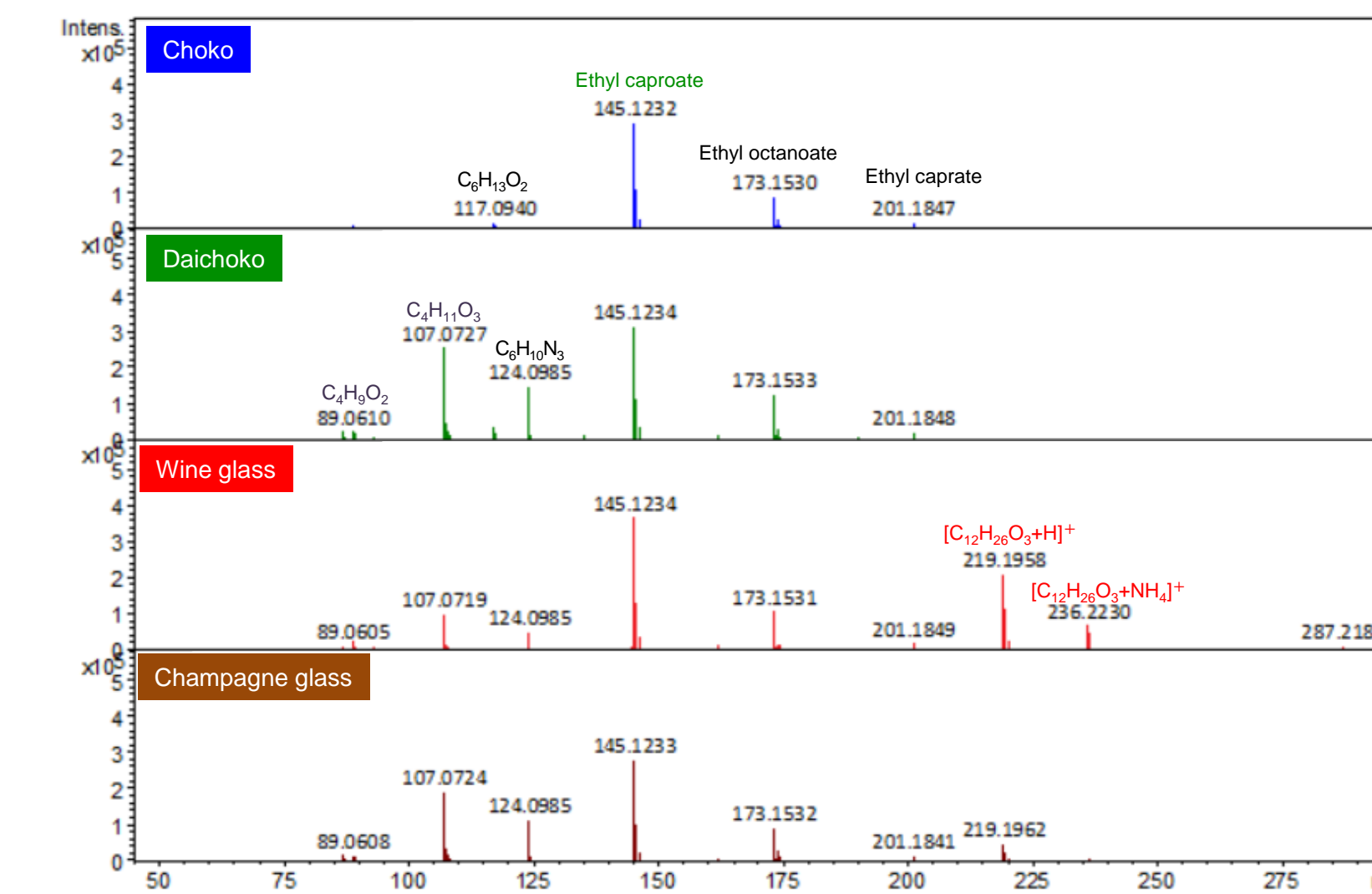


Fig. 8 Averaged mass spectra of Sake in the four types of cup at 1-2 min using Volatimeship DART-MS

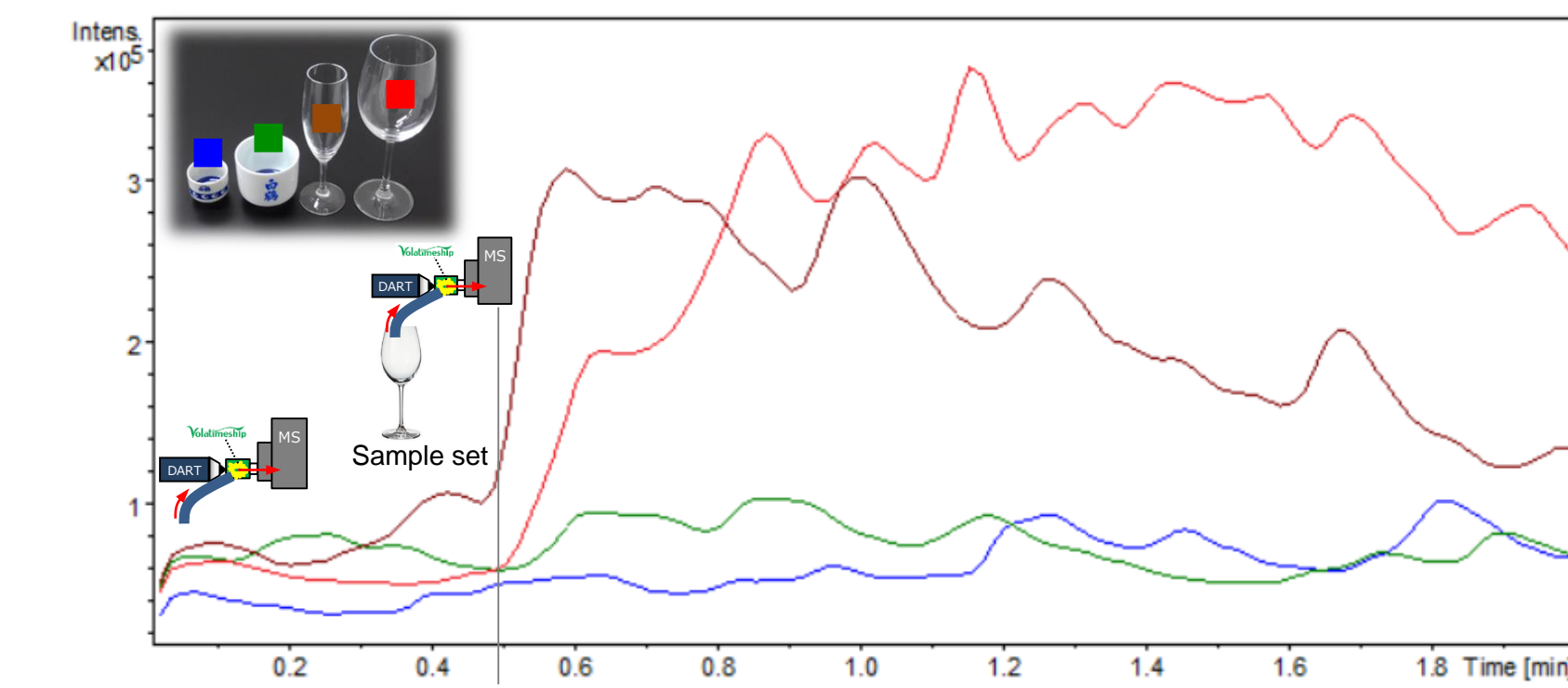


Fig. 9 EIC ( $m/z$  219.19±0.01) of Sake in the four type of cups using Volatimeship DART-MS

## Conclusion

GC/MS is powerful method for qualitative analysis of fragrance, but, unknown peaks not consistent with spectral library were extremely difficult to analyze. Volatimeship DART-MS could be gave us valuable clue to elucidate chemical structure of unknown compounds, since it enables analysis elemental composition by using accurate mass spectra.

An introducing device for volatile compounds (Volatimeship) combined with DART-MS could be useful for objective evaluation of the duration and change of scent, since it enables continuous detection of the change in fragrance intensity directly from sake in cups.

Additionally, this analysis method could be useful for visualizing the sensory evaluation.

When you drink Sake, especially HAKUTSURU DAIGINJO, we do recommend using wine glass in order to enjoy its fragrance !

